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PHARMACOLOGICAL ANTIOXIDANT STRATEGIES AS THERAPEUTIC INTERVENTIONS FOR COPD

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Abstract

Cigarette/tobacco smoke/biomass fuel-induced oxidative and aldehyde/carbonyl stress are intimately associated with the progression and exacerbation of chronic obstructive pulmonary disease (COPD). Therefore, targeting systemic and local oxidative stress with antioxidants/redox modulating agents, or boosting the endogenous levels of antioxidants are likely to have beneficial effects in the treatment/management of COPD. Various antioxidant agents, such as thiol molecules (glutathione and mucolytic drugs, such as N-acetyl-L-cysteine and N-acetylcysteine, erdosteine, fudosteine, ergothioneine, and carbocysteine), all have been reported to modulate various cellular and biochemical aspects of COPD. These antioxidants have been found to scavenge and detoxify free radicals and oxidants, regulate of glutathione biosynthesis, control nuclear factor-kappaB (NF-kappaB) activation, and hence inhibiting inflammatory gene expression. Synthetic molecules, such as specific spin traps like α -phenyl-N-tert-butyl nitron, a catalytic antioxidant (ECSOD mimetic), porphyrins (AEOL 10150 and AEOL 10113), and a superoxide dismutase mimetic M40419, iNOS inhibitors, lipid peroxidation inhibitors/blockers edaravone, and lazaroids/tirilazad have also been shown to have beneficial effects by inhibiting the cigarette smoke-induced inflammatory responses and other carbonyl/oxidative stress-induced cellular alterations. A variety of oxidants, free radicals, and carbonyls/aldehydes are implicated in the pathogenesis of COPD, it is therefore, possible that therapeutic administration or supplementation of multiple antioxidants and/or boosting the endogenous levels of antioxidants will be beneficial in the treatment of COPD. This review discusses various novel pharmacological approaches adopted to enhance lung antioxidant levels, and various emerging beneficial and/or prophylactic effects of antioxidant therapeutics in halting or intervening the progression of COPD.

Keywords

Tobacco smoke; antioxidants; oxidants; redox; glutathione; thiols; Nrf2; lipid peroxides; protein carbonylation; Chronic Obstructive Pulmonary Disease

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1. Introduction

A high-oxygen environment with exogenous pollutants/toxicants constantly being inhaled through the inhaled breath, significantly challenges the lung tissue. Due to its very high blood supply and large surface area, the lung is highly susceptible to oxidative stress mediated injury. In addition, the lung epithelium is constantly exposed to oxidants generated endogenously during metabolic reactions (e.g., from mitochondrial electron transport activation of phagocytes), as well as to inhaled oxidants in the ambient air, including ozone, nitrogen dioxide, diesel exhaust, and cigarette smoke.

Generations of reactive oxygen species (ROS), such as superoxide anion ($O_2^{\bullet-}$) and the hydroxyl radical ($\bullet OH$) are directly associated with the oxidative modification of proteins, lipids, carbohydrates and DNA. Oxidative stress causes cell damage, cell necrosis, apoptosis, autophagy, remodeling of extracellular matrix and blood vessels, endothelial dysfunction, inactivation of antiproteases, premature cellular senescence, elevated mucus secretion, steroid resistance, unfolded protein response, cell proliferation, epigenetic changes, autoimmunity, and skeletal muscle dysfunction. Furthermore, these oxidants also influence inflammatory responses in the lungs through activation of transcription factor nuclear factor kappaB (NF- κ B), mitogen-activated protein kinases (MAPK) signal transduction pathways, chromatin remodeling, and transcription of proinflammatory mediator genes [1–5]. These processes are intricately associated with the progression of chronic obstructive pulmonary disease (COPD) [3–8]. Hence, in light of oxidative burden and its consequences in pathogenesis of COPD, this review is focused on various antioxidant strategies based on various pharmacological, chemical and functional aspects of thiols, spin traps, lipid peroxidation and protein carbonylation, and redox sensor inhibitors, enzyme mimetics, synthetic antioxidants, and redox sensors (Table 1).

2. COPD causes and pathogenesis

COPD is a major and increasing global health problem and is the fourth most common cause of death in the developed countries. It is a disabling condition associated with progressive breathlessness with slow decline in lung function. COPD will account for over six million deaths per year and is predicted to increase from the sixth to the third leading cause of death by 2020 worldwide. It is the disease associated with oxidative stress and inflammaging (inflammation and accelerated decline in lung function) when body's antioxidant defense gradually weakens. Patients with COPD experience a poorer quality of life, suffer from comorbidities associated with cardiovascular, muscular (skeletal) and neurological problems. These comorbidities are associated with increased oxidative stress.

Cigarette smoke is the major risk factor for the development of COPD. Cigarette smoke contains an estimated 10^{15} – 10^{17} oxidants/free radicals and ~4700 different chemical compounds, including reactive aldehydes and quinones, per puff [9–11]. Other noxious environmental gases/particles such as aldehydes/carbonyls, NO_2 , SO_2 , and particulate matters, as well as exposure to second hand tobacco smoke, and smoke derived from burning of biomass fuel can also cause oxidative stress and trigger inflammatory responses in the lungs of a susceptible individual.

COPD can be classified into four classes of severity based on lung function [GOLD Guidelines] [1–2, 4–6]. Emphysema, chronic bronchitis with airway obstruction, and small airways disease are the distinct phenotypes of COPD, but most patients show a combination of different overlapping phenotypes. Emphysema is characterized by destruction of the alveolar septa, loss of elastic recoil, airspace enlargement, and hence loss of gas diffusion capacity. Chronic bronchitis affects the larger airways by airway inflammation (recruitment of neutrophils, macrophages, and other immune-inflammatory cells), goblet cell hyperplasia

and mucus hypersecretion. Apart from decline of lung function, COPD patients experience chronic sputum production (mucus hypersecretion), coughing, and often dyspnoea. Small airways disease mainly affects the bronchioles featuring airway inflammation and metaplasia of Clara cells. The pathogenesis of COPD involves several processes, such as oxidative stress, pulmonary, and systemic inflammation, protease/antiprotease imbalance, breakdown in immunity (autoantibody production), apoptosis, autophagy, vascular and extracellular matrix remodeling, impaired tissue repair, alteration of cell proliferation, and cellular senescence/premature aging. All of these processes are implicitly associated with systemic and lung oxidative stress [2] (Fig.1).

3. Cigarette smoke and oxidants

ROS are virtually produced by all of the cells as a result of metabolism within the mitochondria and additionally by inflammatory cells and phagocytes, such as macrophages and neutrophils, as well as during the immuno-inflammatory response against a pathogen. NADPH (nicotinamide adenine dinucleotide phosphate) oxidase enzyme complex, present in phagocytes and epithelial cells, xanthine/xanthine oxidase system, myeloperoxidase, and the heme peroxidases are the main generators of ROS and have been found to be elevated in COPD patients [12–14].

Similarly, nitric oxide synthase generates reactive nitrogen species (RNS) in the form of nitric oxide (NO). NO can combine with $O_2^{\bullet-}$ to form a more damaging peroxynitrite anion ($ONOO^-$) leading to formation of nitrotyrosine. It has now been reported that the physiological action of an oxidant depends on the source of its generation. For example, oxidants derived from NADPH oxidase mainly participate in cell signaling, while those produced excessively by mitochondria induce cell damage/death [15].

Production of ROS by phagocytes can be enhanced by oxidants present in cigarette smoke leading to the release of inflammatory mediators. Some of these mediators are chemotactic in nature and lead to the recruitment of neutrophils and other inflammatory cells into the lungs. Smokers and patients with COPD have greater migration of macrophages and neutrophils into their lungs as compared to non-smokers. These cells can generate ROS via the activation of NADPH oxidase complex system, leading to further augmentation of oxidative stress in lungs of smokers and patients with COPD [2–5].

3.1. Cigarette/tobacco smoke as a source of oxidants and carbonyls

Cigarette smoke contains more than 10^{15-17} oxidant/free radical molecules per puff and over 4,700 highly reactive chemical compounds including aldehydes/carbonyls [9], this adds to the oxidant burden in current smokers. The gas phase of cigarette smoke contains relatively short-lived $O_2^{\bullet-}$ and NO, which immediately react to form the highly reactive and toxic peroxynitrite $ONOO^-$. A very high diffusion coefficient of $ONOO^-$ allows it to diffuse over a greater distance, thus causing more tissue damage in lung interstitium/microenvironment at the site of generation. The tar phase of cigarette smoke contains organic radicals, such as long-lived semiquinone and quinone radicals, which can rapidly react with molecular oxygen in a redox dependent manner leading to generation of various ROS, such as $O_2^{\bullet-}$, $\bullet OH$, and H_2O_2 [10–11]. The aqueous phase of cigarette smoke condensate (simulated as bronchoalveolar fluids of smokers) may undergo redox recycling for a considerable period of time in the epithelial lining fluid (ELF) of smokers [10]. The tar phase is also an effective metal chelator, wherein iron is chelated to produce tar-semiquinone and tar- Fe^{2+} , which can generate H_2O_2 continuously [10].

3.2. Cigarette smoke, lipid peroxidation and protein carbonylation

Systemic and lung formation of protein carbonyls (aldehyde protein adducts) in response to cigarette smoke-derived lipid peroxides/carbonyls (aldehydes) have been implicated in the pathogenesis of COPD. The levels of lipid peroxidation products, such as 8-isoprostane/ F_2 -isoprostanes are increased in smokers and in patients with COPD [16–17]. Furthermore, the levels of these lipid peroxidation products have been correlated with airway obstruction [16]. 4-hydroxy-2-nonenal (4-HNE) is a specific, highly reactive, and diffusible end-product of lipid peroxidation. 4-HNE forms adducts with cysteine, lysine and histidine residues of proteins which are referred as protein carbonyls. Increased 4-HNE-modified protein levels (protein carbonyls) are present in airway and alveolar epithelial cells and endothelial cells, and in neutrophils in smokers with airway obstruction compared to subjects without airway obstruction [17]. The increased level of 4-HNE adducts was inversely correlated with lung function measured as FEV_1 , suggesting a role for 4-HNE in the pathogenesis of COPD. Also, α,β -unsaturated aldehydes (4-HNE and acrolein) contained in cigarette smoke impose oxidative stress and elicit IL-8 release in pulmonary cells through MAPK activation [18].

3.3. Depletion of antioxidants

Systemic antioxidant capacity is decreased in smokers and in patients with COPD, which is reflected by decreased plasma antioxidants and protein sulfhydryls [19]. This occurs transiently during smoking and resolves rapidly after smoking cessation. However, low grade oxidative stress still persists in ex-smokers who develop COPD. The depletion of antioxidant capacity and antioxidants is due to the increased release of ROS from peripheral blood neutrophils and monocytes implying the role of oxidants in the pathogenesis of COPD. Several other studies have shown the deficiency of antioxidants in both plasma and lungs of patients with COPD (reviewed in [2, 4–5, 20]). It has also been recently reported that antioxidants, such as uric acid, glutathione (GSH), vitamin E, and ascorbate are decreased in smokers, and was associated with the severity of COPD exacerbation [21]. Cigarette smoke irreversibly modifies GSH levels to GSH-conjugates in airway epithelial cells and epithelial lining fluids leading to antioxidants deficiency and injurious lung response [3–6, 20]. Cigarette smoking is shown to block the protective expression of Nrf2/antioxidant response element (ARE) pathway in peripheral mononuclear cells of young heavy smokers favoring inflammation [22].

4. Therapeutic strategies for COPD

Tissue injury and inflammation due to ROS are common to many disease pathologies including COPD and this similarity provides a unique antioxidants therapeutic opportunity wherein numerous lung diseases may be gradually treated by an agent that can suppress either the generation of ROS or can neutralize such species or both. Perhaps this might be true at the onset of the disease progression and pathogenesis for antioxidants to be considered as pre-empting agents. Nevertheless, overwhelming evidence suggest that oxidative/carbonyl stress is implicitly involved with the pathogenesis of COPD. A rationale approach for the treatment of COPD would, therefore, be to consider antioxidant intervention, not only to neutralize the excessive oxidative stress, inhibit peroxidation of lipids, and the subsequent inflammatory response, but also to identify the source of oxidants and overwhelm their generation. This can be achieved by two approaches, either by increasing the endogenous antioxidant enzyme defenses or by enhancing the non-enzymatic defenses through dietary or pharmacological means. Several small-molecular weight antioxidant compounds that target oxidant signaling, or quench cigarette smoke-derived oxidants and reactive aldehydes are currently being tested clinically and pre-clinically [4, 20, 23]. Antioxidant agents, such as thiol compounds/donors and analogs (GSH and mucolytic drugs, such as N-acetyl-L-cysteine (NAC), nalcystelyn (NAL), erdosteine,

fudosteine, and carbocysteine lysine salt), and ergothioneine have all been reported to scavenge/detoxify free radicals/oxidants, increase intracellular thiol levels, control NF- κ B activation, and consequently inhibit inflammatory gene expression. Other compounds include dietary antioxidants, superoxide dismutase (SOD) mimetics, and synthetic redox modulating agents. Several strategies either to increase the expression/activity of the antioxidant enzymes or to mimic their function by enzyme mimetics have been developed. In this review, the beneficial and/or prophylactic effects of a variety of antioxidants are discussed. These antioxidant molecules may have the potential to be used as pharmacological therapeutic agents in the management and treatment of COPD. The pros and cons for targeting oxidative stress by antioxidants in treatment of COPD are given in Table 2.

Dietary antioxidants, such as antioxidant vitamins and carotenoids (vitamin C, tocopherol, β -carotene), and natural products-containing dietary polyphenols/flavanoids, and other active compounds (curcumin, resveratrol, green tea-catechins, quercetin), lycopene, acai, ginkgo biloba, polyunsaturated fatty acids/omega-3 fatty acid, tocotrienols, alpha-lipoic acid, and apocynin are also important in management of oxidative stress imposed by cigarette smoke in smokers and patients with COPD [4–5]. However, the beneficial effects of these dietary products are not part of this review, but have been discussed elsewhere [4–5].

5. Antioxidant enzyme mimetics and spin traps

Antioxidants, such as SOD, catalase, and glutathione peroxidase play an important role in the neutralization of free radicals in the cells. However, their expression and activities are altered in the lungs of smokers and patients with COPD [1–6]. Alterations in antioxidant enzyme activity can be overcome by small compounds possessing catalytic properties. These compounds can mimic the activity of a larger enzyme-based molecule, which are known as enzyme mimetics.

5.1. SOD mimetics

Three classes of manganese (Mn)-containing SOD mimetics have been developed. The first class includes several macrocyclic Mn(II)-pentaazamacrocyclic ligands, such as M40401, M40403, and M40419 [24–25]. Mn(III)-metalloporphyrins, such as AEOL-10113 and AEOL-10150 form the second class of SOD mimetics [26–27], and Mn(III)-salen complexes forms the third class. Since the ‘Salens’ are also reported to have catalase-like activity, they can therefore neutralize H_2O_2 in the cells. In addition, Mn(III)-salen complexes can also decompose ONOO^- [28]. Therefore, Salens appear to have a non-selective nature of action, a property attributed to the multivalent state in which they can exist. In an animal model of lung disease, M40419 has been shown to significantly decrease the markers of oxidative stress in the lungs, and to block the development of emphysema [24]. Since studies with recombinant SOD treatment has shown to prevent neutrophil influx into the lungs and decrease IL-8 release induced by cigarette smoking [29], it is therefore, possible that these compounds, which possess antioxidant enzyme properties may be used as novel antioxidant/anti-inflammatory drugs in COPD. In an ovalbumin challenged model of airway inflammation, AEOL10113 was found to inhibit both airway inflammation and airway hyperreactivity [26]. In another study in rats, AEOL10150 was shown to inhibit cigarette smoke-induced lung inflammation and decreased lipid peroxidation as well as generation of ONOO^- [27].

The metalloporphyrin-based catalytic antioxidant, MnTE-2-PyP (Manganese (III) Meso-Tetrakis-(N-methylpyridinium-2-yl) porphyrin) has broad antioxidant properties, and has been reported to scavenge superoxide, lipid peroxides, ONOO^- , and H_2O_2 [30–32].

Inflammation and injury induced by a wide variety of factors, such as radiation, bleomycin, or lipopolysaccharide exposure is also decreased by MnTE-2-PyP [33–34]. MnTE-2-PyP also reduces NF- κ B signaling by modulating the redox environment of the cell [33]. The ability of MnTE-2-PyP to affect such a wide variety of different disease states has been attributed to its ability to alter inflammatory redox cell signaling [35].

Another SOD/catalase mimetic EUK-189 when administered in rats subjected to heat stress, improved hepatic functions, redox status, decreased oxidative injury, and DNA binding activity of redox response transcription factor NF- κ B [36]. EUK-189 also preserved the redox potential in aged heat-stressed rats by maintaining GSH/GSSG ratios to normal control levels. Furthermore, EUK-189 also increased the expression of stress-responsive protein, heat shock protein 72 (HSP70) in rats subjected to heat stress [37]. Overexpression of SOD1 attenuates cigarette smoke- and elastase-mediated emphysema in mice [38]. Interestingly, a recent observation that sustained lung activity particularly in pulmonary vascular bed of a novel chimeric protein SOD2/3 can be achieved by intratracheal administration in rats [39]. Therefore, these compounds appear to have potential for therapeutic use in COPD.

The third type of SOD, extracellular superoxide dismutase (ECSOD or SOD3) is highly expressed in lungs and vessels [40] and is located in the extracellular matrix (ECM), the junctions of airway epithelial cells, the surface of airway smooth muscle, and the lining of blood vessels of the lung. Since SOD3 scavenges $O_2^{\bullet-}$, it may therefore, play an important role in oxidative lung damage and injury. It has been reported that acute loss of SOD3 in adult mice causes death, whereas overexpression of SOD3 in animals exposed to hyperoxia reduces mortality, suggesting an essential role of SOD3 for survival against the lethal effect of hyperoxia [41–42]. In cigarette smoke/elastase induced emphysema mouse model, SOD3 was shown to protect the lung against pulmonary emphysema, which was attributed to the reduction of oxidative ECM fragmentation and oxidative posttranslational modifications of elastin fragments (leading to autoantibody production) in the lung [43]. Similarly, a recent study showed that SOD3 down modulated CS-induced oxidative stress in mouse macrophages [44]. Hence, SOD3 may be more effective than other SODs in management of emphysema or more severe COPD phenotype.

Smoking and COPD were also found to increase sputum levels of extracellular superoxide dismutase due to adaptive response [45]. Polymorphisms in the SOD3 gene were associated with emphysema, but not COPD susceptibility, highlighting the importance of phenotype definition in COPD genetic studies [46–47]. Furthermore, administration of SOD mimetic significantly attenuated the elastase-induced emphysema in both wild-type and SOD3 knockout mice, and the level/activity of SOD3 was decreased in the emphysematous mouse lung. As mentioned above, SOD3 was also reported to protect against oxidative fragmentation of ECM, such as heparin sulfate and elastin, thereby attenuating lung inflammatory response and emphysema [43]. Therefore, the development of pharmacological mimetics to replenish/augment SOD3 specifically in the lung (matrix) may have a therapeutic potential in the intervention of COPD/emphysema. Furthermore, SOD3 therapy can be combined with other therapeutic approaches in management of COPD.

5.2. Glutathione peroxidase (GPx) mimetic

Ebselen, a selenium-based organic complex, has been found to mimic the activity of glutathione peroxidase (GPx). Since ebselen can also increase the efficiency of GSH, it can thus be used as therapy against oxidative stress and inflammation. Ebselen possesses strong antioxidant activity and has been reported to have strong neutralizing effect against peroxynitrite radical [48]. In human leukocytes exposed to peroxynitrite, ebselen was found

to inhibit the activation of NF- κ B/AP-1 and expression of pro-inflammatory genes expression. *In vivo* activity of ebselen has been demonstrated by virtue of its ability to prevent LPS-induced airway inflammation [49–50]. However, its protective effect against cigarette smoke induced lung inflammation is yet to be reported. BXT-51072 and BXT-51077, which are low molecular weight, orally active organoselenium GPx mimetic have shown to possess the peroxide neutralizing capabilities and regulate inflammation by preventing the activation of inflammatory mediators [51]. The mechanism underlying these protective effects is thought to be via inhibition of transcription of the inflammatory mediators.

5.3. Peroxynitrite decomposition catalysts

Peroxynitrite decomposition catalysts, unlike other enzyme mimetics, possess catalytic activity against peroxynitrite. They are iron containing porphyrin complexes and are similar in structure to AEOL10150 and AEOL10113. In neonatal rat intestinal ischemic-reperfusion model, administration of FeTMPyP, a peroxynitrite decomposition catalyst, provided limited protection by reducing P-selectin expression, systemic NO production, neutrophil infiltration in ileum and lipid peroxidation in both lungs and ileum; and preserved intestinal antioxidant capacity [52]. Another peroxynitrite decomposition catalyst, FP15, decreased ischemic injury in rats and improved cardiac function and survival of mice in a chronic model of doxorubicin-induced cardiotoxicity [53]. The efficacy of peroxynitrite decomposition catalysts has been reported *in vivo* in various animal models of disease associated with peroxynitrite generation [54]. However, it remains to be seen whether or not this class of antioxidants is also effective in a smoking model for COPD associated with high levels of peroxynitrite generation.

5.4. Spin traps

Spin traps are chemical agents, which can quench free radicals and are as such used experimentally for studying reactions involving free radicals. On trapping free radicals, spin traps form a stable end product suitable for measurement. Most spin traps have a nitron- or nitroxide-nucleus and are derivatives of these moieties. Although spin traps have been extensively used for *in vitro* studies, their therapeutic *in vivo* effects have also been investigated in inflammation using α -phenyl-N-*tert*-butyl nitron [55]. One of the difficulties of studying with earlier spin traps was their extremely small half lives and their generation of dangerous \cdot OH on decay. This disadvantage has however, been overcome by introduction of electron withdrawing moieties around the core pyrroline ring [56]. Spin traps containing nitron- core have been shown to possess strong antioxidant properties, and their therapeutic effects have been investigated in Alzheimer's and Parkinson's diseases [57]. Isoindole-based nitrones [58] and azulenyl-based nitrones [59] are already in vogue and one such agent STANZ has been reported to have strong antioxidant properties and can inhibit lipid peroxidation *in vitro*. A phenyl-based nitron spin trap NXY-059 has been tested in acute ischemic stroke. These compounds have also been shown to impart therapeutic benefits in a number of animal models of lung disease. Recent studies have suggested that inhibition of NO generation via iNOS inhibition by various chemical inhibitors [N(6)-(1-iminoethyl)-L-lysine (L-NIL), G-nitro-L-arginine-methyl ester or L-NAME or using the knockout mouse model resulted in attenuation of emphysema [60–61]. This effect may be due to the removal of NO so that ONOO will not be formed. It is possible that selective inhibition of iNOS along with supplementation of other antioxidants may provide a strategy in management of severe COPD. Further investigations are required to develop these compounds (spin traps and iNOS) for long-term use in cigarette smoke/oxidative stress mediated chronic lung diseases including COPD.

5.5. Nrf2 activators

Nuclear factor erythroid 2 p45-related factor 2 (Nrf2) is a basic-leucine zipper (b-ZIP) transcription factor present in the cytoplasm [62]. It plays a pivotal role in cellular defense against electrophiles and ROS present in cigarette smoke. In response to various factors, such as oxidative, carbonyl and electrophilic stresses, Nrf2 detaches from its cytosolic inhibitor, Kelch-like ECH-associated protein 1 (Keap1), and translocates into the nucleus. Upon entering in the nucleus, Nrf2 binds to ARE of target genes along with other binding factors and cofactors leading to an induction of stress response genes [63–65]. Most of the antioxidants and phase II cytoprotective genes, such as heme oxygenase-1 (HO-1), NAD(P)H/quinone oxidoreductase 1 (NQO1), glutamate-cysteine ligase, glutamyl cysteine synthase, glutathione peroxidase, and several members of the glutathione S-transferase family are regulated by Nrf2 [63, 66–67].

Studies have also shown increased susceptibility of Nrf2 null mice to cigarette smoke-induced emphysema compared with wild-type mice [68–69], suggesting the protective role of Nrf2. Furthermore, Nrf2 appears to protect against pulmonary hyperoxia/bleomycin injury and ovalbumin challenge in mice, presumably by upregulating the transcription of lung antioxidant defense enzymes. It has been shown that reduction of Nrf2 protein levels in pulmonary macrophages and in lungs of patients with COPD occurs via loss of Nrf2 positive regulator DJ-1 and posttranslational modifications of the Keap1–Bach1 equilibrium [70–72]. There is a possibility that polymorphisms in the promoter region of Nrf2 gene may affect the phenotype in COPD. The levels of Nrf2 protein are decreased in lungs of patients with COPD, and this decrease was associated with reduction/oxidative posttranslational modifications and degradation of histone deacetylase 2 (HDAC2) [73–74].

HDAC2 is known to be post-translationally modified by carbonylation and S-nitrosylation on cysteine residues [75–78]. S-nitrosylation of HDAC2 is known to occur at Cys 262 and Cys 274 which does not affect its deacetylase activity [79]. A recent study has shown that in alveolar macrophages obtained from patients with COPD, S-nitrosylation of HDAC2 at Cys-262 and Cys-274 is increased, and this modification abolishes its GR-transrepression activity, and renders corticosteroid insensitive for its efficacy [80]. Furthermore, activation of Nrf2 by sulforaphane denitrosylates HDAC2, and subsequently restores dexamethasone sensitivity in alveolar macrophages. It is also known that HDACs inactivation by oxidative/carbonyl stress may antagonize deacetylase activity [81]. Aldehydes, such as 4-HNE and acrolein alkylate (carbonylate) HDAC2 by covalent modification at two conserved cysteine residues, corresponding to Cys(261) and Cys(273) [81]. Hence, it remains to be seen whether S-nitrosylation and thiol alkylation which occurs on these cysteine residues would also be reversed by Nrf2 activation, though oxidative/nitrosative posttranslational of HDAC2 is subject to ubiquitination and proteosomal degradation [77]. Furthermore, Nrf2 and HDAC2 both are carbonylated in response to CS and in patients with COPD. These dual modifications on each molecule (cysteine residues) may cause specific steric hindrance and post-translational modifications can lead to proteosomal degradation. Nevertheless, it may be possible to reverse the carbonylation and S-nitrosylation of HDAC2 by pharmacological means (via activation of thioredoxin reductase and carbonyl/aldehyde reductases) so that they can function effectively in early stages of COPD and overcome steroid resistance.

Compounds which are potent activators of Nrf2, may buffer the antioxidant potential in the lung against cigarette smoke, particularly in patients with COPD, can thus be developed for their therapeutic application. Alternatively, novel compounds which are potent activators of Nrf2 or which stabilize Keap1/DJ-1/Maf proteins can be developed. Therapeutically, it has been shown that activating Nrf2 with the compound 1[2-Cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO)-imidazolidine can reduce CS- and LPS-induced inflammation providing a novel therapeutic strategy for COPD [65, 82]. Such compounds will augment

the antioxidant potential in the lung against cigarette smoke, particularly in patients with COPD. Furthermore, it may be possible to combine the strategy of Nrf2 activators with other currently available therapeutic options (e.g. phosphodiesterase 4 inhibitors, bronchodilators, and steroids) in management of COPD. However, one has to keep in mind whether there is a polymorphism in the promoter region of Nrf2 gene in susceptible smokers and patients with COPD.

Various small molecule activators of Nrf2 signaling are currently being investigated for their anticancer and anti-inflammatory properties (both lung cancer and COPD are associated to cigarette/tobacco smoke exposure). A wide variety of dietary and synthetic compounds that function as potent inducers of ARE-regulated gene expression have been shown to exert chemopreventive activities, e.g., various dietary and synthetic compounds, such as sulforaphane [82], dithiolethione [83], curcumin and caffeic acid phenethyl ester (CAPE) [84] have been reported as potent inducers of ARE regulated genes.

Chalcones or chalconoids (1,3-diphenyl-2-propen-1-ones) and Michael acceptors are important groups of naturally occurring compounds of antioxidant and chemoprotective agents [85]. Chalcones have been found to have relevant physiological properties, due to their ability to bind to proteins related to cell apoptosis and proliferation [86]. Recently, it has been reported that chalcones can impart anti-inflammatory effects due to the inhibition of the NF- κ B pathway [87–88]. Furthermore, chalcones have also been reported to activate Nrf2/ARE pathway, thus inducing phase II detoxifying enzyme expression [89]. Currently, various derivatives of chalcones are being developed for improving the anti-inflammatory and anti-cancer properties of chalcones, which may find potential therapeutic role in COPD [62].

6. Redox sensors: Enzymatic and non-enzymatic

The intracellular redox state is a dynamic system and is constantly influenced by the energy status of the cell. Various external and internal factors can alter the redox state of the cell. The most common redox sensors of the cell include the NADP/NADPH, NAD⁺/NADH, and glutathione systems (GSH/GSSG). Since the relative concentrations of the reduced versus oxidized components of these redox couples determine the overall redox state of the cell, the effect of any external oxidative stress is detected by these redox sensors. While GSH is the most abundant cytosolic thiol redox sensor, the NADP/NADPH system acts as a buffer against mitochondrial redox shifts. In addition, NADP/NADPH is associated as a cofactor with several enzymatic reactions and with lipid metabolism.

6.1. Thioredoxin

Thioredoxin (Trx) and redox effector factor-1 (Ref-1) belong to oxidoreductase family of redox sensors. During oxidative stress, Trx, which is bound to proteins, such as hepatopoietin [90] and the apoptosis signal regulating kinase (ASK-1) [91], as heterodimeric complexes are released from these complexes liberating the proteins [90]. This leads to the multimerization of ASK-1 and dimerization of hepatopoietin. In the dissociated form, Trx reduces a key thiol group within cys62 of the p65/NF- κ B subunit in the nucleus leading to transcriptional activation [92]. Concomitantly, the prevailing oxidative stress activates free ASK-1, leading to the activation of the pro-inflammatory p38MAPK and JNK pathways [3–5, 20]. The two pathways discussed above are redox sensitive, which is supported by the observation, wherein inhibition of Trx (may lead to reducing environment in the nucleus) with MOL-294 (a small molecular weight inhibitor of Trx), blocks activation of both pro-inflammatory NF- κ B and AP-1-dependent transcription. Inhibition of NF- κ B activation by MOL-294, is also accompanied by diminished neutrophil influx and TNF- α production [93]. Similarly, Ref-1, another redox sensor, on inhibition by PNRI-299, leads to the inactivation

of AP-1 pathway [94]. Furthermore, *in vivo* administrations of both MOL-294 and PNRI-299 in a murine ovalbumin challenge model of asthma have been shown to reduce various clinical parameters [94–95]. On the contrary to above findings, Trx, overexpression of Trx-1 or treatment of mice with recombinant human Trx attenuates cigarette smoke-mediated oxidative stress and pulmonary emphysema [96]. This may be due to its direct intra- or extracellular antioxidant property rather than its nuclear effect. However, this molecular effect in cigarette smoke-mediated lung damage, inflammatory response, and in pathogenesis of COPD remains to be investigated.

A family of tri- and tetra-oligopeptides derived from the canonical CxxC motif of the Trx active site and a modified CxC motif has been recently synthesized [97]. These constitute the Trx-mimetic compounds, which are N- and C-terminal-blocked peptides that consist of two cysteine residues that flank the two-amino-acid CxxC motif (CB4 and CB6) or the single-amino-acid CxC motif (CB3). These Trx-mimetics have been reported to upregulate various redox sensitive processes in the cell [97]. Thus, upregulation of Trx by various synthetic small molecules may be an important strategy employed against oxidative stress and abnormal inflammatory responses occurring in patients with COPD.

6.2. Peroxiredoxins

Peroxiredoxins (Prdx), are members of a superfamily of selenium-independent peroxidases. Prdx exerts a protective role through peroxidase activity against H_2O_2 , peroxynitrite and phospholipid hydroperoxides [98–99]. Depending on the number of active Cys residues, mammalian Prdx falls into typical double-cysteine (Prdx1–Prdx4), atypical double-cysteine (Prdx5), and single-cysteine (Prdx6) classes. Prdx6 is highly expressed in the lung tissue, alveolar epithelial type II cells, bronchial Clara cells, and alveolar macrophages [100–101]. Prdx6 protects against oxidative stress-induced lipid peroxidation and apoptosis and regulate cellular signaling [102]. Therefore, it appears that Prdx6 is an important protective enzyme that counteracts oxidant stress in the lung. The important protective role of Prdx in defense against oxidative stress was demonstrated in a study involving *PRDX1* gene knockout mice [103]. In another study, it was hypothesized that targeted disruption of *PRDX6* would lead to enhanced susceptibility to cigarette smoke (CS)-mediated lung inflammation and/or emphysema in mouse lungs [104]. However, this study revealed that targeted disruption of Prdx6 did not increase lung inflammation, but was associated with increased antioxidants, suggesting a critical role of lung Prdx6 and several compensatory antioxidant mechanisms during acute CS-induced adaptive response. Furthermore, it was found that the protective effect of Prdx6 was lost in chronic CS exposure leading to pulmonary emphysema. Prdx acts as a substrate for sulfiredoxins (Nrf2-dependent), another enzyme important in redox antioxidant pathways. Strategies to increase the expression of Prdx or pharmacological amplification by small molecules may, therefore, be beneficial in the treatment of COPD.

6.3. Glutaredoxin

Glutaredoxins (Glrx) are thiol disulfide oxido-reductases, which possess antioxidant and catalytic functions closely associated with GSH. Glrx1 is a potential redox modulatory protein regulating the intracellular, as well as extracellular homeostasis of glutathionylated proteins and GSH in human lung. Glrx1 is implicated in regulation of pro-inflammatory NF- κ B signaling and chromatin modifications [105–106]. In a cross-sectional study, it has been demonstrated that Glrx1 was mainly expressed in alveolar macrophages, extracellular fluids and sputum supernatants, and the levels of this enzyme were decreased in COPD patients [107]. Glrx1 mRNA and protein expression was decreased in alveolar epithelial cells exposed to CS along with decreased activity and increased protein S-glutathionylation [108]. In the same study, irreversible oxidation and carbonylation of recombinant Glrx1 by CS and

acrolein was also demonstrated, which was associated with its reduced enzyme activity. Interestingly, overexpression of Glrx1 attenuated protein S-glutathionylation induced by CS and increased cell survival [108]. Tracheal epithelial cells of mice deficient in Glrx1 were found to be more sensitive to CS-induced cell death with corresponding increases in protein S-glutathionylation [108]. Thus, CS can modulate Glrx1 at both the gene expression level and also by modifying Glrx1 enzyme activity. A recent study has shown that Glrx1 regulates cigarette smoke-mediated lung inflammation via S-glutathionylation and differential modulation of I κ B-kinases in mice [105]. Hence, pharmacological intervention or strategies to increase Glrx1 gene expression and modulate its enzyme activity would have potential in the regulation of cigarette smoke-mediated lung inflammation in COPD.

7. Small molecule thiol antioxidants

7.1. N-acetyl-L-cysteine (NAC)

NAC is an acetyl derivative of the amino acid, cysteine, and is a strong reducing agent (Fig. 2). NAC has been used as a mucolytic agent and reduces mucus viscosity, thereby improving muco-ciliary clearance. In the gastrointestinal tract, NAC is deacetylated into cysteine and also serves as precursor of GSH in the cells. Its ability to reduce disulfide bond makes it a good reducing agent and also allows it to neutralize oxidant species. Within the cells, NAC reduces cystine into cysteine, and may thus serve as an important mechanism for elevation of intracellular GSH *in vivo* in lungs. Oral NAC attenuates elastase-induced pulmonary emphysema in rats implicating its possible role in the management of COPD [109].

Clinical studies regarding the beneficial effects of NAC and other thiols in patients with COPD has yielded mixed results (Table 3) [110–122]. While a Cochrane systematic review showed a significant reduction of 0.79 exacerbations per patient per year compared with placebo, a 29% decrease in number of exacerbations, whereas small-scale trials failed to demonstrate any clear clinical benefits [113]. However, a few meta-analyses have shown a small, but significant clinical benefit in patients with COPD [115, 123].

Considering that GSH plays an important role in the control of oxidative/carbonyl stress in COPD, NAC has mainly been used, although with varying success, to enhance lung GSH in patients with COPD [116]. A randomized, double-blind, placebo controlled phase II trial, with a 6-month oral dose of 600 mg NAC, twice daily, was found to significantly reduce various plasma and BAL fluid oxidative biomarkers in smokers [124]. Another study involving oral administration of NAC (600 mg twice daily for 2 months) was shown to rapidly reduce oxidant load in the airways of stable COPD patients [120], and was associated with a reduction in bronchial mucus hypersecretion and a decline in FEV₁, and COPD exacerbations [114].

NAC has a beneficial effect on systemic oxidative stress as oral administration of NAC was found to increase the quadriceps endurance time of severe COPD patients [125]. In contrast, another multicenter study using NAC delivered by metered-dose inhalers in patients with chronic cough did not show any beneficial effect on well-being, sensation of dyspnea, cough, or lung function [126]. Although there is a body of evidence suggesting beneficial effects of NAC in COPD patients, it is not clear whether NAC is used as a mere maintenance therapy [110]. However, a phase III multicenter trial, Bronchitis Randomized on NAC Cost-Utility Study (BRONCUS), has supported this notion [127]. Although BRONCUS study showed no effect on decline in FEV₁, a reduction in overinflation and exacerbation in patients with severe COPD not treated with inhaled glucocorticoids was observed [111]. In a recent study, using an infection model of A549 alveolar epithelial cells infected with the influenza virus, NAC was found to inhibit induction of MUC5AC,

interleukin (IL)-8, IL-6, and TNF-alpha [128]. NAC was also shown to inhibit the replication of the influenza virus in the same study.

In view of the above, and due to conflicting reports on the beneficial effects of NAC, further studies are required using NAC at higher doses (1200 or 1800 mg/day, or even higher doses) or by using other thiol agents with greater bioavailability in order to observe any clinical benefits on lung function, reduced exacerbation rate, and improved health status/quality of life. Overall, NAC can be perceived to play a therapeutic role in the management of COPD due to its ability to increase intracellular GSH, along with its known mucolytic properties. Therefore, NAC may be more likely to be of benefit in subjects with mucus hypersecretion and in other COPD phenotypes, which requires further clinical studies regarding NAC as a therapeutic alternative against COPD and its pulmonary emphysema phenotype.

7.2. N-acetylcysteine (NAL)

NAL, a lysine salt of NAC, is also a mucolytic and antioxidant (reducing) thiol compound (Fig 2). While NAC is acidic in solution, NAL has a neutral pH in solution and can be aerosolized into the lung without significant side effects [129]. In a comparative study involving NAL and NAC, it was found that both of the compounds enhanced intracellular GSH in lung alveolar epithelial cells and inhibited the release of H_2O_2 and $O_2^{\bullet-}$ from human blood-derived polymorphonuclear leukocytes in smokers and patients with COPD [129]. This study also revealed that NAL could inhibit ROS generation by serum-opsonized zymosan stimulated human polymorphonuclear neutrophils in a fashion similar to NAC. NAL could also act as an anti-inflammatory agent, as shown in a study where NAL was shown to inhibit oxidant-mediated IL-8 release in monocytes. Therefore, NAL is an antioxidant and anti-inflammatory agent, which may have a potential therapeutic benefit for COPD treatment. Its ability to be aerosolized adds to its therapeutic advantage. For example, the lung deposition of NAL was shown to be effective, when given via a metered dose inhaler formulation in healthy subjects [130]. Befitting from its therapeutic advantage, a clinical trial using NAL for treatment of COPD is needed.

7.3. Procysteine and GSH-esters

Procysteine (L-2-oxothiazolidine-4-carboxylate or cysteine 1-2-oxothiazolidine-4-carboxylic acid) is a cysteine-relieving compound that increases the intracellular cysteine levels, and has greater bioavailability than NAC. This thiol compound is well-tolerated and has been shown to increase mitochondrial levels of GSH in alveolar type II cells [131]. A recent study by Hodge *et al* showed that cigarette smoke-induced changes to alveolar macrophage phenotype activated M1 (proinflammatory; regulation of antigen presentation) or alternatively activated M2 (poor antigen presentation; improved efferocytosis) markers and function (efferocytosis and proinflammatory cytokine production) are improved by treatment with procysteine in mouse lung. It is concluded that the increased efferocytosis and availability of GSH in response to procysteine may be useful as adjunct therapy for improving macrophage function in COPD and in susceptible smokers [132].

Glutathione esters, particularly GSH monoethyl esters, can increase the GSH levels of the cells by cleavage of ester bond (an ethyl group esterified to glycine). GSH esters have been shown to increase GSH levels in the lungs of rats and *in vitro* in epithelial cells; however, this compound can be cytotoxic, and variation in the uptake levels of GSH has been shown in various cellular models [133]. Furthermore, it may be cautious to note that therapeutic strategies involving GSH or its analogs or thiol compounds must be carefully designed keeping in mind the species barrier, bioavailability, half-life of the analogs, and generation of toxic byproducts by the analogues, as well as interference with metabolic and signaling

pathways in a cell or tissue. One such alternative for GSH drug delivery will be via nanomedicine/liposome-encapsulated form.

7.4. N-isobutyrylcysteine (NIC)

One of the disadvantages of using NAC is its low bioavailability, due to its greater first pass hydrolysis in biological systems. Therefore, a drug having similar effects as NAC but with greater bioavailability might prove to be a more effective treatment for chronic bronchitis/COPD. One such compound is NIC, which is also a thiol molecule with lesser first pass hydrolysis and bioavailability as high as 80% compared to NAC [134]. However, therapeutic evaluations of NIC against exacerbations in chronic bronchitis have revealed that NIC did not do better than the placebo [129]. A similar non-effective result was reported in a study in patients with COPD [134].

7.5. Erdosteine

Erdosteine is a thiol antioxidant having mucoactive properties (Fig 2), and the ability to reduce bacterial adhesiveness. This compound was introduced as a mucolytic agent for the treatment of chronic pulmonary diseases. Erdosteine breaks the disulfide bonds of mucus glycoproteins, affecting the physical properties of the mucus, thus leading to increased cough clearance [135]. In addition, erdosteine has been reported to have antioxidant, anti-inflammatory, and antibacterial activity.

In a randomized, placebo-controlled clinical study, 'Equalife' involving oral administration of 300 mg erdosteine twice daily for 8 months [121] showed significant reduction in exacerbations compared to the placebo group. The same study also demonstrated that patients receiving erdosteine showed significant improvements in their quality of health without any adverse effect on lung function. In another clinical study involving administration of erdosteine 300 mg twice a day for 7 – 10 days improved the symptoms and reduced the length of time for the disease in patients with acute exacerbation of chronic bronchitis and COPD [136]. Long-term treatment of stable COPD patients with erdosteine reduced the rate of hospitalization and acute exacerbation. Furthermore, erdosteine has been reported to impart health benefits in patients suffering from repeated, prolonged or severe exacerbations of COPD [136–137]. Dal Negro *et al.* showed that erdosteine at a dose of 600 mg/day proved effective in significantly reducing ROS levels in peripheral blood of stable COPD patients who are current smokers, together with reduction in levels of some chemotactic proinflammatory cytokines (IL-6 and IL-8) in their bronchial secretions [138]. Recently, the anti-inflammatory properties of erdosteine were confirmed in a study involving COPD subjects, wherein by its ability to reduce the proinflammatory eicosanoids levels [139]. Hence, this compound may be a choice as another antioxidant and mucolytic agent in management of COPD.

7.6. Carbocysteine

S-carboxymethylcysteine (carbocysteine or S-CMC) is a blocked thiol derivative of amino acid; L-cysteine (2). Oral preparations of carbocysteine both as S-CMC and its lysine salt (S-CMC-lys) are available. The lysine salt is activated in the gastrointestinal tract post-cleavage of the lysine residue to yield the active drug S-CMC. Carbocysteine is a mucoactive drug with reported *in vitro* free radical scavenging and anti-inflammatory properties. While mucolytic drugs, such as NAC and erdosteine bear free sulphhydryl (thiol) groups, through which they split glycoprotein bonds in mucous, carbocysteine differs in structure and mechanism of action from other commonly available mucolytics. The mucus produced under the influence of carbocysteine, has increased sialomucin content. Sialomucins influence the rheological properties of mucus via the inhibition of kinins, thereby reducing or preventing bronchial inflammation and bronchospasm [140].

In COPD patients treated with S-CMC-Lys for a 6-month period, Carpagnano *et al* demonstrated a significant reduction in exhaled 8- isoprostane and pro-inflammatory cytokine: IL-6, providing an *in vivo* support for the spate of *in vitro* evidences, proving that the drug has anti-inflammatory and antioxidant properties [141]. Furthermore, anti-inflammatory action of carbocysteine has been well documented in models of pulmonary inflammation involving several different cytokine profiles and it has been shown to protect against emphysema induced by cigarette smoke in rats [142]. In carrageenan-induced pleurisy, oral administration of S-CMC-lys has been shown to attenuate neutrophil recruitment in acute IL-1 β -induced airway inflammation and neutrophil, macrophage and eosinophil migration into the pleural space [143]. Carbocysteine attenuated mucus glycoproteins hypersecretion and reduced inflammatory cells, free radical and elastase activity in the bronchoalveolar lavage fluid of rats with sulfur dioxide-induced airway inflammation have been reported [144].

Carbocysteine is shown to enhance both *in vivo* and *in vitro* muco-ciliary clearance velocity, particularly in patients with chronic bronchitis with slow clearance before treatment [140]. Since carbocysteine has the potential to reduce bacterial respiratory tract infections in COPD, it has been suggested that it may act through the inhibition of pathogen adherence to cells. *In vitro* studies involving treatment with carbocysteine have demonstrated a significant reduction in adherence of *Moraxella catarrhalis* (this bacteria causes exacerbations of COPD) to the pharyngeal epithelial cells, of both healthy subjects and those with chronic bronchitis, when compared to placebo treated group [145]. This observation has been further corroborated by a similar observation that, carbocysteine significantly reduced attachment of *Streptococcus pneumoniae* to pharyngeal epithelial cells taken from healthy subjects by direct *in vitro* treatment of the cells with the drug [146]. Reduction in frequency of common colds including infections and associated exacerbations in COPD patients on treatment with carbocysteine have been attributed to its ability to decrease ICAM-1 expression in the respiratory tract [147].

There are reports regarding the clinical use of carbocysteine to treat COPD patients with mixed results on alterations in lung function and exacerbation rate (Table 3) [122, 147–155]. A recent study, particularly the PEACE study involving 709 Chinese COPD subjects, has examined the effect of over three year period of treatment with carbocysteine (250 mg t.d.s) focusing on the rate of COPD exacerbations. This study has revealed that COPD patients whom were treated with carbocysteine experienced fewer numbers of exacerbations per year [122]. Although very few side effects have been reported for carbocysteine, over-dosage may cause mild side-effects, such as gastric discomfort. More systematic studies and understanding the detailed mechanism of action of carbocysteine may uphold the candidature of carbocysteine as a major therapeutic agent for treatment of respiratory diseases including COPD. Furthermore, a longer use of carbocysteine may be more effective for preventing acute exacerbations in patients with COPD. Overall, clinical studies have revealed that carbocysteine is well-tolerated, has mucoactive properties (mucolytic) and can also reduce bacterial adhesiveness. Currently, carbocysteine is considered to be a useful muco-regulatory agent for the treatment of COPD that provides symptomatic relief to some patients, along with resolute sputum production, and may reduce exacerbation rates. Carbocysteine may be choice as compared to NAC, or other available thiols in management of COPD and its exacerbations.

7.7. Fudosteine

Fudosteine, [(–)-(R)-2-amino-3-(3-hydroxypropylthio)] propionic acid (Fig 2), has been used in the treatment of chronic respiratory diseases, such as bronchial asthma, chronic bronchitis, pulmonary emphysema, COPD, and bronchiectasis as a mucoactive agent [156–157]. Fudosteine acts by increasing cysteine levels of the cells and has greater

bioavailability than NAC. Fudosteine downregulates MUC5AC gene expression thus inhibits mucin hypersecretion [156]. Although, little is known about its mode of action, fudosteine has shown some promising potential in treatment of patients with COPD [158]. These findings suggest that fudosteine may be useful in controlling oxidative/carbonyl stress-related mucus secretion states in patients with asthma, bronchiectasis or COPD. Fudosteine (500 mg/kg, p.o.) significantly augmented the output volume of respiratory tract fluid in rabbits and increased chloride ion concentration in bronchoalveolar lavage of rats [159]. Fudosteine also reduced the activation of p38 MAPK and ERK pathways [156]. Fudosteine has also been shown to inhibit peroxynitrite-induced cellular alterations in lung by direct scavenging of this free radical [160]. Further studies are required clinical efficacy of fudosteine in treatment of COPD.

7.8. Ergothioneine

Ergothioneine (2-mercaptohistidine trimethylbetaine) is a naturally occurring antioxidant found in most plants and animal tissues (Fig 2) [161]. Chemically, L-ergothioneine corresponds to the betaine of 2-thio-L-histidine. In aqueous solution, the tautomeric-2-thioimidazole exists predominantly in the thione form. The compound is known to be formed via hercynine from histidine, methionine, and cysteine in microorganisms. Ergothioneine inhibits the peroxynitrite-dependent nitration of nitrotyrosine [162], and also inhibits oxidative DNA repair and cell death [163]. Ergothioneine also inhibits the formation of xanthine and hypoxanthine (a precursor of xanthine), which are shown to be activated in patients with COPD [4–5]. The role of ergothioneine on S-nitrosoglutathione catabolism and generation of vasodilatory NO has been shown [164].

Interestingly, ergothioneine increases cellular tolerance/availability of NAC. The role of ergothioneine in inhibition of oxidative stress-mediated signal transduction mechanisms involved in cellular inflammatory responses has been shown in lung epithelial cells. Ergothioneine inhibits oxidative stress- and TNF- α -induced NF- κ B activation and IL-8 release in alveolar epithelial cells [165]. Thus, ergothioneine may be a potential antioxidant/anti-inflammatory agent to inhibit the chronic inflammatory response, which occurs in the development of chronic inflammatory lung diseases including COPD. However, no documented evidence are available to attest this in management of COPD.

8. Lipid peroxidation and protein carbonylation inhibitors/blockers

8.1. Edaravone (MC-186)

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a widely acclaimed and potent free-radical scavenger and exhibits its antioxidant ability by inhibiting lipid peroxidation [166–167]. Interestingly, edaravone can also inhibits protein carbonylation by direct carbonyl-scavenging mechanism [168]. Protein carbonylation and carbonyl stress via aldehydes and advanced glycation end-products occur in several pulmonary diseases including COPD and hence it may be the direct of prevention strategies [2, 17, 169–170]. Since edaravone has protective effects on both hemispheric embolization and transient cerebral ischemia, it has therefore been clinically tested to treat acute brain infarction in Japan [171–173]. In a study in rat model of ischemia/reperfusion injury, Ito et al. [174], has demonstrated that edaravone ameliorated the lung injury induced by intestinal ischemia/reperfusion. In the same study [174], edaravone was found to decrease infiltration of neutrophils, membrane lipid peroxidation and downregulate expression of IL-6 mRNA in the lungs, leading to a significant reduction in mortality. Edaravone also shown to be protective in acute pancreatitis-associated lung injury by suppression of IL-6 and TNF- α [175]. Furthermore, it has been shown that edaravone suppressed bleomycin-induced acute pulmonary injury in rabbits [176] and paraquat-induced lung injury and fibrosis in rats [177], and suppresses

fibrosis in tight-skin and bleomycin-induced mouse models of systemic sclerosis [178]. This suggests an important role of edaravone in suppressing skin and lung fibrosis. The protective effect of edaravone was demonstrated by its ability to inhibit lung injury and fibrosis via decreasing lipid peroxidation and the enhancing prostaglandin E2 production in the experimental murine system. Another analog MCI-186 (3-methyl-1-phenyl-pyrazolin-5-one) is shown to have protective effects against free radical damage, but shown have biphasic effects on deterioration of pulmonary fibrosis in mice [179]. Edaravone seems to be interesting molecule which inhibits protein carbonylation, which occurs in response to cigarette smoke and in patients with COPD. Hence, further studies are required to demonstrate the therapeutic efficacy of edaravone in management of COPD and its symptoms.

8.2. Lazaroids (U75412E or tirilazad mesylate): a nonglucocorticoid 21-aminosteroid

Lazaroids (21-aminosteroids, U75412E or tirilazad mesylate) are a group of nonglucocorticoid analogues of methyl-prednisolone which are able to penetrate hydrophobic regions of the cell membrane, specifically to prevent peroxidation of membrane lipids [180–181]. The protective efficacy of lazarooids has been reported in many animal models of lung injury, such as injury by endotoxin [181], ischemic/reperfusion [182] and tobacco smoke [183]. The findings from the above studies have revealed that lazarooids may exert their protective effect by various mechanisms, inhibition of lipid peroxidation being the main. In a cigarette smoke-induced lung injury model, it was found that aerosolized lazarooids could inhibit the formation of free radicals and also inhibit release of TNF- α in alveolar macrophages [184–185]. In another study, it was found that lazarooid U74389G was able to protect against hyperoxia in rat type-II pneumocytes [186]. Lazarooid, U74006F was reported to protect against endotoxin mediated lung injury in sheep and protected against antigen-induced bronchoconstriction and bronchoalveolar eosinophilia in allergic sheep [187–188], and sepsis-induced acute lung injury in minipigs [189]. Furthermore, lazarooids were found to attenuate oxidant-induced lung injury by inhibiting the mRNA expression for E-selectin, P-selectin, ICAM-1, and VCAM-1 [190]. However, studies are required to evaluate the efficacy of lazarooids as therapeutic strategy in COPD, particularly in severe COPD where steroid resistance occurs.

9. Conclusions

Oxidative stress is known to occur in the pathogenesis of COPD. Targeting oxidative stress with pharmacological antioxidants or boosting the endogenous levels of antioxidants is likely to be beneficial in the treatment and management of COPD. Several small molecule antioxidant compounds have been investigated in clinical and pre-clinical trials. Antioxidants, such as thiol molecules/donors, spin traps, antioxidant enzyme mimetics, and inhibitors of oxidative stress and lipid peroxidation and protein carbonylation blockers have potential to be used in treatment/management of COPD, as well as in different COPD phenotypes. However, the clinical trials so far have been unsuccessful to clearly define (1) the type of antioxidant, (2) regimen, and (3) time period of treatment in acute exacerbations and pathogenesis of COPD. This is perhaps due to lack of understanding of the pathophysiology of COPD and subtle differences in COPD phenotypes. It is, therefore, important to identify the main source(s) of oxidant in a particular phenotype and accordingly choose a particular antioxidant therapy or regimen for a given COPD phenotype.

Current therapy using antioxidants for COPD is symptomatic, e.g. thiol-based therapy is mainly mucolytic (clearance of mucus, thereby minimizing the bacterial/viral load). However, it is possible that antioxidant therapy becomes therapeutic based on future studies involving novel compounds in amelioration of COPD. Furthermore, antioxidant compounds may also enhance the efficacy of glucocorticoids by quenching oxidants and aldehydes in

COPD patients. Although thiol antioxidant treatments have shown promising effects in targeting ROS and oxidant-mediated cellular alterations, development of novel wide-spectrum small molecule antioxidants with a good bioavailability and potency are needed in clinical trials for COPD. Antioxidant therapy may affect important outcomes in COPD, such as overcoming steroid resistance, mucus hypersecretion, inflammation and extracellular matrix remodeling. Furthermore, the effects of a combination of various antioxidants along with thiols, spin traps, lipid peroxidation/protein carbonylation inhibitors/blockers, or enzyme mimetics is an interesting proposition worth investigating in patients with COPD. Antioxidants (e.g. thiols and other molecules) may be combined with anti-inflammatories/PDE4 inhibitor/Sirtuin1 activator, bronchodilators, steroids, antibiotics, and statins. Furthermore, novel antioxidant strategies can be used as supplementing/pre-emptying agents in management of COPD, as well as in susceptible smokers.

Highlights

Cigarette smoke causes oxidative stress in COPD.

Antioxidants are likely to have beneficial effects in management of COPD.

Thiols, enzyme mimetics, spin traps, and porphyrins are therapeutic agents.

Antioxidants have pharmacological effects in inhibiting cellular processes in COPD.

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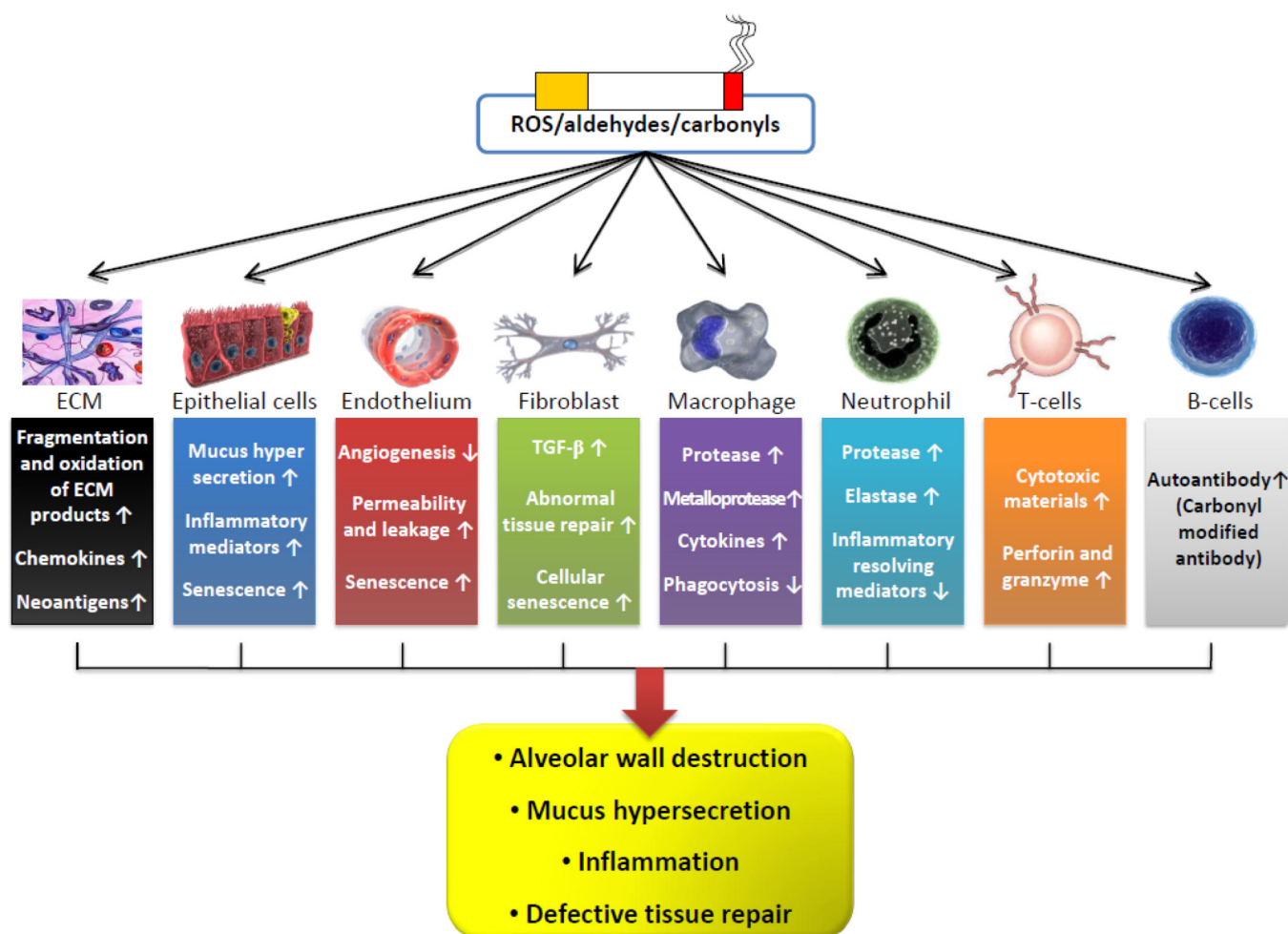


Figure 1.
Chemical structures of thiol antioxidants

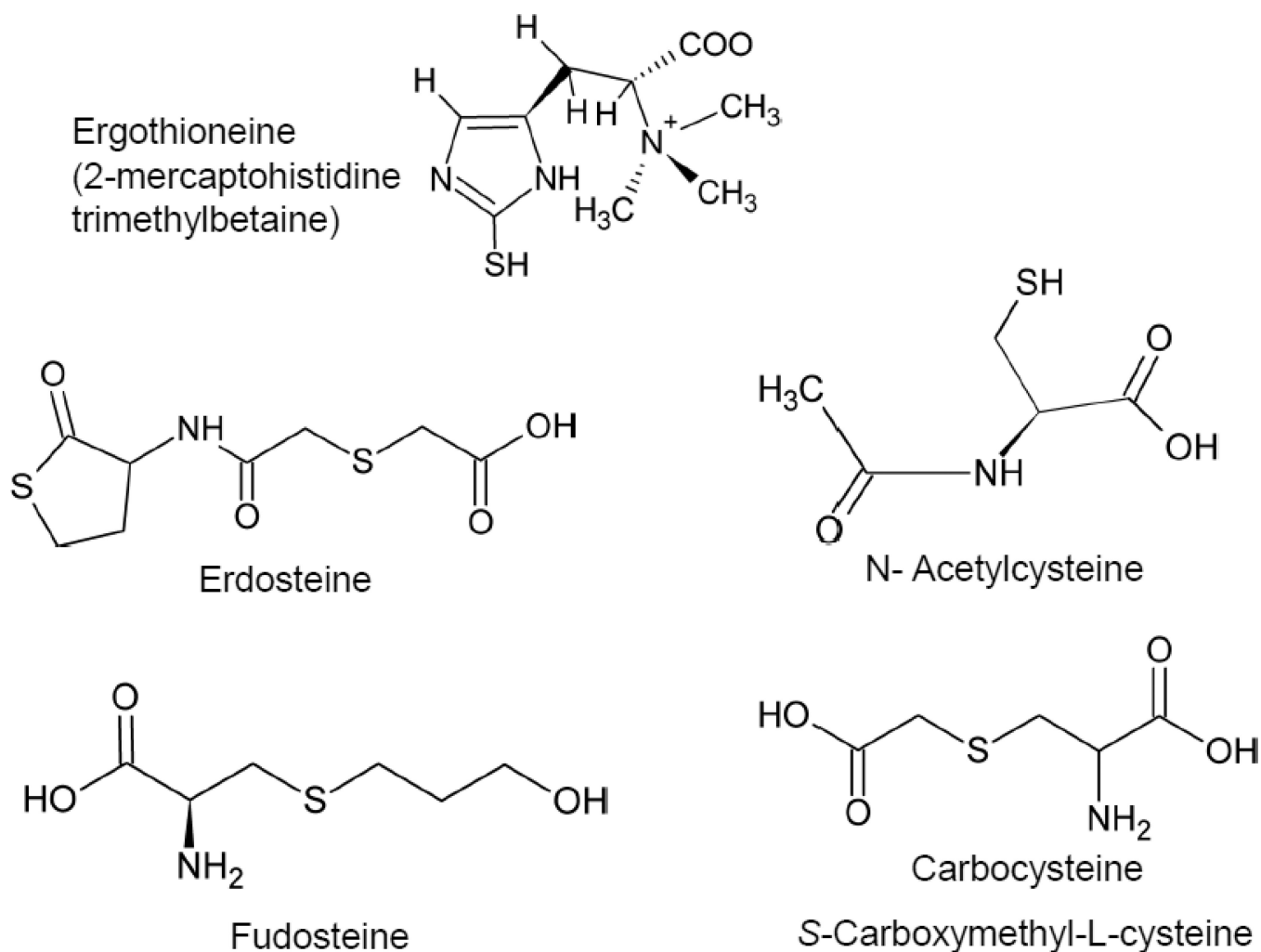


Figure 2. Consequences of oxidative stress in pathogenesis of COPD

Inflammatory response is mediated by inhaled and/or cellular oxidants and carbonyls. These oxidants activate alveolar macrophages, neutrophils, eosinophils, and epithelial cells, leading to augmented endogenous ROS generation, redox imbalance, activation of redox-sensitive transcription factors, mucus hypersecretion, extracellular matrix and small airway remodeling. ROS can trigger several cellular and molecular events which are involved in pathogenesis of COPD. These cellular and molecular events can be exploited as targets for pharmacological antioxidant strategies in treatment of COPD.

Table 1

Antioxidant therapeutic agents in COPD

Antioxidant compound	
<i>Thiol compounds</i>	N-acetyl-L-cysteine (NAC) N-acetylcystein (NAL) N-isobutyrylcysteine (NIC) Glutathione esters S-carboxymethylcysteine (Carbocysteine) Erdosteine Fudosteine Thioredoxin Procysteine Ergothioneine
Inducers of glutathione biosynthesis	
<i>Nrf2 activators</i>	CDDO-imidazolide Sulforaphane Chalcones (1,2-diphenyl-2-propen-1-ones)
Glutathione peroxidase and SODs	
<i>Mimetics</i>	Salen compounds M40419, M 40403, and M40419 Manganese-metaloporphyrins, such as AEOL-10113 and AEOL-10150 Metaloporphyrins (MnTBAP) MnTE-2-PyP EUK compounds (EUK-189) Ebselen BXT-51072 (ALT-2074), and BXT-51077
Lipid peroxidation/protein carbonylation inhibitors/blockers	Edaravone (MC-186) Lazaroids/tirilazad (U75412E or tirilazad mesylate)
Other antioxidants/enzymes and oxidant scavengers	Nitron spin traps (NXY-059, STANZ) Peroxynitrite decomposition catalysts (FeTMPyP, FP15) Porphyrins Glutaredoxins Peroxiredoxins
Polyphenols, natural products, and dietary agents	Polyphenols/flavanoids/natural compounds (Curcumin, resveratrol, quercetin, sulforaphane, lycopene, Acai, Apocynin, omega-3-fatty acid, Vitamin D)

Table 2

Pros and cons of targeting oxidants with antioxidants in COPD

Antioxidant	Pros	Cons
<i>Thiol compounds</i>	<ul style="list-style-type: none"> • Potent mucolytic • Mild effects on exacerbation rates 	<ul style="list-style-type: none"> • Lack of bioavailability • Cause bronchoconstriction • Form thiyl/cysteine radicals
<i>Nrf2 activators</i>	<ul style="list-style-type: none"> • Protection seen in animal models • Induce a variety of phase II genes/enzymes • Clinical studies are required 	<ul style="list-style-type: none"> • Dramatically degraded in severe COPD GOLD stages • Carbonylation/oxidation of Nrf2/Keap1 • Prophylactic-potent inducer is needed
<i>Targeting ROS using SOD mimetics</i>	<ul style="list-style-type: none"> • Protection seen in animal models • Inhibit ECM remodeling • Clinical studies are required 	<ul style="list-style-type: none"> • May impair phagocytosis • Alter endogenous redox homeostasis
<i>Lipid peroxidation/protein carbonylation inhibitors/blockers</i>	<ul style="list-style-type: none"> • Inhibit lipid peroxidation chain reactions • Clinical studies are required 	<ul style="list-style-type: none"> • Poor bioavailability • Low presence at the site of microenvironment

Table 3

Clinical trials conducted for the efficacy of thiol antioxidants in smokers and COPD

Clinical Trial	Antioxidant	Study Aim	Outcome	References
BRONCUS	NAC	Effect of NAC on FEV ₁	No effect. No change on decline in FEV ₁ . A reduction in lung overinflation in patients with severe COPD without inhaled glucocorticoids. Decrease of exacerbation if NAC and Inhaled corticosteroids combined.	[110–111]
Systematic Cochrane review of 23 randomized, controlled trials	NAC (2 months of oral therapy)	Effect of NAC and antibiotics on number of days of disability	No difference in lung function. Significant reduction in days of disability (0.65 day per patient per month) and 29% reduction in exacerbations	[112–113]
Systematic Cochrane review of randomized, controlled trials; 11 of 39 retrieved trials	NAC	Use of validated score to evaluate the quality of each study	9 trials showed prevention of exacerbation and 5 of which addressed improvement of symptoms compared with 34.6% of patients receiving placebo	[114]
Meta-analysis of published trials	NAC	Assess possible prophylactic benefits of prolonged treatment	23% decrease in number of acute exacerbations	[115]
-	NAC (600 mg/d, 5 days and 600 mg, 3/d, 5days)	Effect of NAC on GSH and cysteine	Increase of GSH at day 5 and cysteine (plasma) at day 5	[116]
-	NAC (600 mg once a day for 12 months)	Effect of NAC on H ₂ O ₂ and TBARS in EBC	No change in TBARS levels Reduce H ₂ O ₂ levels	[117]
-	NAC (600 mg/d, 7days)	Effect of NAC on FEV ₁ , breathlessness	No difference compared to placebo group	[118]
-	NAC (600 mg/d)	Effect of NAC on cytokine and exhaled breath condensate (ECD)	Decrease IL-8 and ECP level in NAC group	[119]
-	NAC (600 mg x 2/d x 2 months)	Effect of NAC on H ₂ O ₂	Decrease H ₂ O ₂ level in NAC group	[120]
EQUALIFE studies	Erdosteine-thiol compound, 300mg b.i.d for months	Effect on exacerbation rate, hospitalization, lung function and quality of life	Decreased exacerbations and fewer days in hospital. No loss of lung function and improvement in health-related quality of life	[121]
-	Erdosteine 300 mg twice a day for 7 – 10 days	Effect on COPD symptoms during exacerbations and hospitalization days	Improvement of symptoms and reduced time of disease in patients with acute exacerbation of chronic bronchitis and COPD	[136–137]
	Erdosteine 600 mg/day	Effect on oxidative stress and inflammation	Reduced ROS levels in peripheral blood of stable COPD patients who are current smokers, together with reduction in levels of IL-6 and IL-8 in bronchial secretions	[138–139]
Small scale UK studies	Carbocysteine	Effect of 2.25–3.00 g carbocysteine daily along with placebo in chronic bronchitis	Heterogeneous results on alterations in FEV ₁ peak flow rate and dyspnea scores	[148–151]
An Italian multi-center, prospective, double-blind RCT involving 662 outpatients with a chronic bronchitis	Carbocysteine	Effect of 2.7 g S-CMC-Lys once daily for 6 months on COPD patients	No significant difference in baseline FEV ₁ between the groups. Mean time to first exacerbation was significantly prolonged and significant	[152]

Clinical Trial	Antioxidant	Study Aim	Outcome	References
			reduction in mean days of acute respiratory illness per patient.	
A double-blind, parallel-group study in the UK in 109 patients with chronic bronchitis over 6 winter months	Carbocysteine	Effect of 750 mg carbocysteine three times daily compared with placebo on peak flow and exacerbation rate.	No significant difference in exacerbation rate. Significant increases in peak flow from baseline in both placebo and intervention groups	[153]
RCTs in Tokyo in 156 patients with COPD over a 12-month period	Carbocysteine	Effect of 1.5 g carbocysteine daily with placebo	No significant differences in severity of COPD. Significant reduction in the number of common colds and reduction in rate of exacerbation	[154–155]
RCTs in Japan involving 142 patients with COPD conducted over a 12-month period	Carbocysteine	Treatment with 500mg carbocysteine three times a day	Consistent reduction in exacerbation frequency. No change in lung function	[147]
PEACE study	Carbocysteine (carbocisteine)	Effect on rate of exacerbations	Long-term (one year) use of carbocysteine (1500 mg/day) produced reduction in numbers of exacerbations in patients with COPD	[122]

BRONCUS = Bronchitis Randomized on N-acetyl-L-cysteine Cost Utility Study, bid= Twice daily; *FEV₁* = Forced expiratory volume in 1 second, TBARS = Thiobarbituric acid reactive substances, *NAC* = N-acetyl-L-cysteine, *EBC* = Exhaled Breath Condensate, ECP: eosinophilic cationic protein, RCT: Randomized placebo-controlled trials.